## RESPONSE TO OFFICE ACTION

### A. Status of the Claims

Claims 1-27 were filed with the case. Claims 2-9, 17-22 and 24-27 were canceled herein as drawn to non-elected subject matter or in view of the instantly elected subject matter. Applicants reserve the right to prosecute canceled subject matter in one or more continuing application(s). Claims 1, 10-16 and 23 are therefore now pending.

The Action stated that Applicants' earlier traversal of the Restriction Requirement was non-persuasive and indicated that claims 3-9 and 14-27 were withdrawn from consideration. In response, Applicants note that a Petition will be filed respectfully requesting consideration of all of the currently pending claims. Specifically, the Petition will note that all of the current claims incorporate the limitation of the promoter sequence according to claims 1. For example, transgenic cells or plants transformed with this sequence are claimed, as are two methods of using this sequence. Claim 1 therefore generically links all of the current claims, and further there is no additional burden in examining all of the current claims. This is because, upon a finding that the sequence of claim 1 is novel and nonobvious, the remaining claims would also necessarily be novel and nonobvious without the need for any searching. Reconsideration is thus respectfully requested.

### B. Claim Objection

Claims 1 and 10 are objected to as directed to non-elected inventions. In response, Applicants note that the claims have been amended herein and that the rejection is now moot. Removal of the objection is thus respectfully requested.

## C. Rejection Under 35 U.S.C. § 112, First Paragraph – Enablement

The Action rejects claims 1-2 and 10-13 for failing to comply with the enablement requirement of 35 U.S.C. § 112, first paragraph. Specifically, the Action appear to acknowledge that enablement has been provided for the full length promoter sequence of SEQ ID NO:90, but asserts that fragments of this sequence with promoter activity or other shorter than full-length promoter sequences are not enabled. Applicants respectfully traverse as set forth below.

### 1. The Specification Enables The Claimed Promoter Fragments

The specification provides detailed teaching fully enabling those of skill in the art to produce the claimed fragments of SEQ ID NO:90 having promoter activity. For example, the Detailed Description of the Invention explains that it is known that non-essential elements can be deleted from a promoter without significantly altering the function of the promoter and that the sequences can be mutagenized generally. It is stated that mutagenesis may be performed in accordance with any of the techniques known in the art, including random as well as targeted approaches. It is further stated that, in targeted approaches, comparisons can be made with other promoters to identify regions involved in promoter activity and confirmation of these putative regulatory regions can be achieved by deletion analysis followed by functional assays using expression of reporter genes. With respect to random approaches, it is stated that deletion mutants can be randomly prepared and then assayed. In this technique, a series of constructs are prepared, each containing a different portion of the clone (a subclone), and these constructs screened for activity. As stated in the specification, activity can be determined by attaching a deleted promoter construct to a selectable or screenable marker and to identify those constructs expressing the marker protein. The smallest segment that is required for activity is thereby identified through comparison of the selected constructs.

The working examples in particular provide the methodology that one of skill in the art would use to generate such fragments. For instance, the specification describes in Example 1 the preparation of target cDNA libraries, including three tassel libraries, and background cDNA libraries prepared from leaf; root, embryo, callus, shoot, seedling, endosperm, culm, ear, and silks.

Example 2 describes the identification of promoter leads. A database of EST sequences derived from the cDNA libraries prepared from various corn tissues was used to identify the genes with the desired expression profile from which promoter candidates were isolated for expression of operably linked DNA sequences in male reproductive tissues. The sequences were also used as query sequences against GenBank databases that contain previously identified and annotated sequences and searched for regions of homology using BLAST programs. Table 1 shows the background clone ID (EST) information and GenBank identifier (gi) information for the ESTs used for subsequent isolation of the promoter sequences shown in SEQ ID NOS: 79-98. The promoter leads were obtained from the library source from Example 1.

Example 3 describes genomic library construction, PCR amplification and promoter isolation. For instance, PCR was used to amplify out promoter sequences for cloning. Detailed isolation procedures used for identifying the promoters are given for numerous different promoters, including that corresponding to SEQ ID NO:90, e.g., clone ID 700354681. As explained in the example, for the isolation of the clone ID 700354681 promoter, SEQ ID NO:62 was used in combination with SEQ ID NO:1 (AP1) in a standard GenomeWalker™ PCR reaction using Expand Hi Fidelity DNA Polymerase. For a nested, secondary PCR reaction, a dilution of the primary reaction is used with SEQ ID NO:63 and SEQ ID NO:2 (AP2) in a standard GenomeWalker™ PCR reaction using Expand Hi Fidelity DNA Polymerase. The

secondary PCR reaction was analyzed by agarose gel electrophoresis and the bands isolated and purified. DNA from individual clones was isolated using the Qiagen Plasmid Mini kit and sequenced using the M13 forward primers and M13 reverse primers. The promoter band was subsequently purified using a Qiaquick gel extraction kit.

Example 5 describes transient assays for analysis of promoter activity in protoplasts and microspores. The promoter fragments were first cloned into expression vectors such as pMON19469, shown in FIG. 1. This plasmid is an expression vector consisting of the following genetic components: P-e35S is the promoter for the 35S RNA from CaMV containing a duplication of the -90 to -300 region; HSP70 intron is the intervening sequence of the maize heat shock protein as described in U.S. Pat. Nos. 5,593,874; GUS: 1 is the coding region for beta-glucuronidase; nos 3' is the termination signal from the nopaline synthase gene; ori-M13 and ori-pUC are origins of replication; AMP is the coding region for ampicillin selection. If a translational start codon of a target promoter is identified, the fragment is cloned into pMON19469 in place of the P-e35S genetic element. If an AUG is not identified, the promoter fragment is cloned into an expression vector modified to enable translational fusions with a reporter gene such as GUS or GFP. The expression constructs prepared were tested in a transient plant assay. Several different assays are described in the example, and are well known in the art generally. For instance, Example 6 describes a transient assay of promoter activity in wheat reproductive tissues and Example 7 describes the use of assays and assay results generally. Example 7 in particular explains that, to test the DNA fragments contained in Example 3 for promoter activity, SEQ ID NOS: 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97 and 98 were assayed by particle bombardment of barley microspores (see Example 5) or particle bombardment of wheat reproductive tissues (see Example 6).

Table 2 gives a summary of transient assay data testing for promoter activity of the DNA fragments containing SEQ ID NOS: 82-98. As explained, a DNA fragment comprising SEQ ID NO:90 was operably linked to the hsp70 intron and the GUS gene to generate the construct pMON449300. GUS activity was detected, with activity detected at a level equal to that seen with the e35S positive control with SEQ ID NO:90.

Example 8 shows testing of promoter for activity during stable plant transformation. Table 3 shows the results, with promoter activity shown in plants. For SEQ ID NO:90 in particular, this sequence was placed upstream of a hsp70 intron/MS2 coat protein gene cassette and put into a plant transformation vector resulting in the constructs pMON42914 and pMON42936. Four of five plants were positive for MS2 coat protein and fourteen of nineteen plants were positive for MS2 coat protein. Anthers from three independent R0 wheat plants were assayed by Western Blot for MS2 coat protein, with one of three plants positive for MS2 coat protein. The results therefore shoed that SEQ ID NO: 90 can act as an anther enhanced promoter in both monocots and dicots.

The foregoing therefore describes in detail the studies one of skill in the art would use to confirm the function for any given promoter or fragment thereof. In order to test a fragment of SEQ ID NO:90, one of skill in the art would merely have to repeat the same studies carried out on the full length sequence. To generate the fragment itself, for example, one would simply need to manually shear the DNA of SEQ ID NO:90 or could use one or more restriction enzymes to cleave the DNA. Only the most basic and routine of experimentation would therefore be required in order to obtain fragments of SEQ ID NO:90 with promoter activity. This is greatly underscored when taking into account that generation of such fragments was well known and routine in the art, as set forth below.

## 2. Generation of Promoter Fragments is Well Known in the Art

It must further be underscored that the generation of promoter fragments is well known in the art. This evidence dispels any doubts that, having been provided SEQ ID NO:90, fragments could be generated containing even substantial deletions with only routine experimentation while still retaining promoter activity. For example, Welsch *et al.* (**Exhibit A**; "Structural and functional characterization of the phytoene synthase promoter from *Arabidopsis thaliana* " *Planta* 216: 523–534 (2003)) describe the creation of multiple deletion fragments of an *Arabidopsis thaliana* phytoene synthase (*psy*) gene promoter. Starting with a full length sequence comprising 1746 nucleotides of the 5' region, truncations were created and tested for expression comprising only 1314, 910, 809, 300 and 196 nucleotides of the 5' upstream region. **Exhibit A** at p. 526, FIG. 1. It was shown that the deletion comprising 1314 nucleotides was "almost indistinguishable" in the pattern of expression. *Id.* at p. 526, 2nd col. While a truncation of 196 nucleotides abolished the responsiveness observed in the full length promoter to some types of light, responsiveness (*e.g.*, promoter activity) was still observed to several types of light. *Id.* Therefore, the authors showed that at least 432/1746 (*e.g.*, 24%) nucleotides could be deleted from the full length promoter with essentially no change in expression and at least 1550/1746 (*e.g.*, 89%) nucleotides could be deleted while still retaining promoter activity.

Similarly, Piechulla *et al.* (**Exhibit B**; "Identification of tomato Lhc promoter regions necessary for circadian expression" *Plant Molecular Biology* 38: 655–662, 1998) describe the deletion analysis of promoters from the cab 1A, cab 1B, cab 8 and cab 11 genes from the tomato light harvesting complex of genes to determine which deletions would affect circadian expression. Deletion constructs were tested comprising from between 1091 and 43, from between 793 and 152, from between 322 and 148 and from between 251 to 119 nucleotides from these promoters, respectively. **Exhibit B**, p. 659, Fig. 5. As explained by the authors, the

"results show that the short 5'-upstream regions are sufficient for a basal mRNA accumulation" and further indicate that upstream sequences are responsible for circadian rhythm. The paper therefore demonstrates creation of fragments from three promoter in which at least 1048/1091 (*e.g.*, 96%), 641/793 (*e.g.*, 81%), 174/322 (*e.g.*, 54%) and 132/251 (*e.g.*, 53%) of the nucleotides of these promoters were deleted while retaining promoter activity.

The foregoing is also illustrated by other studies, such as that of Cho and Cosgrove (**Exhibit C**, *Plant Cell*, 14, 3237–3253, 2002). These authors created deletion fragments in which more than 990 base pairs of an approximately 1428 bp plant promoter sequence designated AtEXP7 were deleted without significantly effecting promoter activity, and creation of even larger deletions while maintaining a reduced promoter activity. See **Exhibit C**, p. 3244, 2nd col. and FIG. 8. The authors also showed deletion of approximately 775 bp from a 1058 bp plant promoter designated AtEXP18 without significantly reducing promoter activity. See *Id.* at FIG. 10. Finally, the authors showed that numerous substitution mutations could be made in a fragment of AtEXP7, while retaining full promoter activity, and in some cases increasing activity. See. *Id.* at FIG. 9 and p. 3245, 2nd col. These studies therefore show that fragments of full length promoter sequences can routinely be made that retain promoter activity.

It would therefore be a straightforward matter for one of skill in the art to generate fragments of SEQ ID NO:90 that retain promoter activity using no more than routine experimentation well known in the art. The art establishes both that these techniques were well known and routine. While some routine screening would be required, "[e]nablement is not precluded by the necessity for some experimentation such as routine screening." *In re Wands*, 858 F.2d at 737. Where the specification "provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed" this does not constitute

undue experimentation. *Id.* (quoting *Ex parte Jackson*, 217 USPQ2d 804, 807 (Bd. App. 1982)). This is underscored by the fact that those of skill in the art knew how to make such changes as of filing date. Therefore, given the detailed teaching in the specification, advanced state of the art and scope of claimed subject matter, compliance with the enablement requirement has been fully demonstrated.

Removal of the rejection under 35 U.S.C. §112, first paragraph, is thus respectfully requested.

**D. Rejections Under 35 U.S.C. § 112, Second Paragraph**

The Action rejects claim 1 as indefinite for reciting that the claimed nucleic acids are "capable of" regulating transcription. Applicants respectfully traverse but note that current claim 1 recites a nucleic acid that regulates transcription of an operably linked nucleic acid. The rejection is therefore moot and removal thereof is thus respectfully requested.

**E. Rejection Under 35 U.S.C. § 102(a)**

(1) The Action rejects claims 1-2 and 10-13 under 35 U.S.C. § 102(b) as anticipated, or in the alternative rendered obvious by, Tacke *et al.* (Genbank X88779). Applicants respectfully traverse.

It is initially noted that Applicants' representative called the Examiner to request a copy of the alignments prepared with respect to the currently cited sequence and sequences below. The Examiner faxed copies of the alignments on August 2, 2006, which have been attached as Exhibits D-F. Applicants thank the Examiner for the time taken in sending these.

As can be seen in the alignment, substantial differences are present between the cited sequence and SEQ ID NO:90. **Exhibit D**. For example, the alignment shows a longest matching segment of 36 base pairs. In contrast, all of the current claims require at a minimum a fragment of SEQ ID NO:90 that regulates transcription of an operably linked DNA sequence. No basis has been provided for concluding that this 36 bp sequence would have promoter activity. This is specifically the burden of the Office to support an anticipation rejection as any rejection must be supported by "substantial evidence" within the record pursuant to the Administrative Procedure Act ("APA"). *See In re Gartside*, 203 F.3d 1305, 1314-15 (Fed. Cir. 2000). In the current case that would include an initial showing on the record that any fragment of a *plant* promoter no more than 36 bp would have activity, putting aside the particular issue of a fragment of SEQ ID NO:90 sharing nucleotides with the cited sequence. The claims therefore cannot be anticipated or rendered obvious.

In view of the foregoing, withdrawal of the rejection is respectfully requested.

(2) The Action rejects claims 1-2 and 10-13 under 35 U.S.C. § 102(b) as anticipated, or in the alternative rendered obvious by, Ainley *et al.*, (Geneseq Accession Nos. AAV6371 and AAV63730 from WO 9856921). Applicants respectfully traverse.

As an initial matter, applicants note that it is believed that the Action inadvertently referred to accession number "AAV6371" rather than accession number AAV63731. The alignments provided confirms this. Applicants' arguments are thus directed to accession number AAV63731.

With respect to the AAV63730 sequence, the alignment reveals substantial differences between the sequences. **Exhibit E**. For example, the alignment shows a region of homology of only 260 nucleotides and a longest matching segment of 32 base pairs. Similarly, an alignment

with AAV63731 yielded the same result, with a region of homology of only 260 nucleotides and a longest matching segment of 32 base pairs. **Exhibit F**.

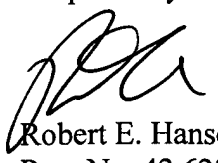
In contrast, all of the current claims require at a minimum a fragment of SEQ ID NO:90 that regulates transcription of an operably linked DNA sequence. There is no scientific basis for asserting that such a fragment would have promoter activity and further no such basis has been shown indicating that any 32 bp fragment would have activity, as is specifically the burden of the Office to support an anticipation rejection. It is well settled that a rejection must be supported by "substantial evidence" within the record pursuant to the Administrative Procedure Act ("APA"). *See In re Gartside*, 203 F.3d 1305, 1314-15 (Fed. Cir. 2000). In the current case that would include, at a minimum, an initial showing on the record that any fragment of a plant promoter no more than 32 bp would have activity, not to mention the particular issue of a fragment of SEQ ID NO:90 sharing any nucleotides with the cited sequence. The claims therefore cannot be anticipated or rendered obvious.

In view of the foregoing, withdrawal of the rejection is respectfully requested.

## CONCLUSION

In view of the foregoing, Applicants respectfully request favorable consideration of this case. The Examiner is invited to contact the undersigned attorney at (512) 536-3085 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



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